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Comparative evaluation of serum CA 125 in oral submucous fibrosis, oral squamous cell carcinoma and healthy controls: A chemiluminescence immunoassay study

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Abstract

Cancer Antigen 125 (CA 125) is also known as mucin 16 or MUC16. It is a glycoprotein present on the surface of normal cells. It appears to be involved in various biological processes including epithelial mesenchymal transition during carcinogenesis. Serum level of CA 125 is utilised to assess the response towards chemotherapy in ovarian carcinoma. The same has also been investigated in other malignancies. Earlier, level of CA 125 has been investigated in saliva in oral squamous cell carcinoma (OSCC) cases. No researches have been conducted so far investigating the serum level of CA 125 in OSCC and potential malignant disorders of oral mucosa. Oral submucous fibrosis (OSF) is a high-risk potential malignant disorder of oral mucosa with numerous systemic alterations. Thus, this study was aimed at investigating serum levels of CA 125 in OSF and OSCC cases. It is a comparative study including three groups viz. group 1 – Patients suffering from OSF, group 2 – Patients suffering from OSCC and group 3 – healthy controls. In each case the serum CA 125 was assessed with chemiluminescence assay. The results obtained were subjected to appropriate statistical methods. We conclude that serum CA 125 may elevate as the normal oral mucosa progresses to the premalignant or a malignant one.

Key words: CA 125, Carcinoma, Chemiluminescence, Fibrosis, Oral, Serum

Introduction

In spite of the availability of advanced treatment modalities for oral squamous cell carcinoma (OSCC), the treatment outcome of these patients is unsatisfactory. In developing countries like India, poor prognosis of OSCC cases is mostly accounted by presentation at advanced stages of disease. Early recognition of changes of impending neoplasia is the only way to improve the prognosis of OSCC cases. Tumour markers help to detect the definite changes occurring during the development of a malignant tumour. Carcinogenesis is an extremely

complex process, which involves expression of various molecules at different stages. The molecules which are expressed during the early events of carcinogenesis can be useful to identify and intercept the malignant process at an early stage. Such molecules can be detected in body fluids or in precancerous/tumour tissues as "tumour markers".

Cancer Antigen 125 (CA 125), also known as mucin 16 or MUC16, is a protein that in humans is encoded by the MUC 16 gene. It is a member of mucin family glycoproteins present in the surface cell wall of normal cells. The antiadhesive property of MUC16 has been suggested to provide a protective barrier for the epithelial surface from bacterial adherence and mechanical injury [1]. CA 125 is also a tumor associated antigen, which is cleaved from the surface of cancer cells and shed into body fluids such as blood and saliva. Recent studies have also shown that CA125 has an important role in epithelial mesenchymal transition during carcinogenesis [2]. The level of CA 125 in serum is shown to be increased with epithelial cancers like ovarian, breast and cervical carcinomas [3,4,5,6]. According to few studies, salivary CA 125 is increased in oral squamous cell carcinoma [7 & 8]. However, there are no studies in academic literature regarding serum level of CA 125 in oral malignancy and premalignancy. Hence, this study was taken up to assess whether serum CA 125 can be used as an early marker of malignancy.

Materials and methods

The research protocol was approved by the institutional ethical committee of Manubhai Patel Dental College hospital and oral research institute, Vadodara, India (MPDC_114/OPATH-28/17). The subjects for the study were selected from the patients coming to outpatient department of Manubhai Patel Dental College and Hospital, Vadodara. The study consisted of three groups, viz. group 1 - 30 cases of Oral Submucous Fibrosis (OSF), group 2 - 30 histopathologcally diagnosed cases of OSCC and group 3 - 30 healthy controls. Informed consent was obtained from each patient for participation in the research. 5ml venous blood was collected with aseptic technique from antecubital vein from each subject in plain blood collection bulb. The blood sample was allowed to clot for one hour. After one hour the serum was centrifuged for 5 minutes at 3000 rpm. Serum CA 125 was quantitatively determined in each case by chemiluminescence immunoassay (CLIA) in automated CLIA machine (Backman Coltar, Access OV Monitor, USA)

Principle and method of chemiluminescence immunoassay (CLIA):

Figure 1 shows schematic representation of the chemiluminescence immunoassay procedure. The assay is a two-site immunoenzymatic (sandwich) assay. In step 1, The serum sample is added to a reaction vessel along with mouse monoclonal anti-CA 125 antigen alkaline phosphatase conjugate and paramagnetic particles coated with a second mouse monoclonal anti-CA 125 antigen antibody. The CA 125 antigen in the sample binds to the immobilized monoclonal anti-CA 125 antigen on the solid phase, while the conjugate antibody reacts with a different antigenic site on the CA 125 antigen molecule. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. During step 2, after washing off the excess, the chemiluminescent substrate Lumi-Phos* 530 is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is directly proportional to the concentration of CA 125 antigen in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

The results thus obtained were statistically analyzed by Mann-Whitney test. For all statistical methods p value ≤ 0.001 was considered significant at 95% confidence interval.

Results and Discussion

Table 1, 2 and 3 show demographic details of patients included in group 1, 2 and 3 respectively. The mean serum CA 125 level in healthy subjects was 7.77 U/ml whereas it was 11.50 U/ml and 19.32 U/ml in OSF and OSCC groups respectively (Table 4, Figure 2). The differences between mean CA125 levels in three groups were statistically analyzed by Mann-Whitney test. The difference between serum levels of CA125 of group 1 (OSF) and group 3 (healthy controls) was found to be statistically significant ($p = 0.001$). Similarly, difference between serum CA 125 levels between group 2 (OSCC) and group 3 (healthy controls) was also found to be statistically significant ($p < 0.001$). However, serum levels of CA125 between group 1 (OSF) and group 2 (OSCC) did not differ significantly ($p > 0.001$).

The functional role of CA 125/MUC16 in cancer is not well understood. In recent studies by Lakshmanan et al it was found that CA 125/MUC16 induce rapid G2/M transition causing increased proliferation in breast cancer cells and stable knockdown of MUC16 in breast cancer cells resulting in significant decrease in the rate of cell growth, tumorigenicity and increased apoptosis [9]. In vitro studies by Comamala and Pinard et al showed disruption of intercellular junctions, enhanced motility, migration and invasiveness in CA 125/MUC16 knockdown cells [2]. Hence, they suggested that CA 125/MUC16 plays a role in epithelial mesenchymal transition, presumably through its interaction with E-cadherin and β-catenin complexes and by modulating EGFR. In current study the serum CA 125 level showed significant increase during the progression from healthy oral mucosa to precancerous mucosa or OSCC. This result is similar to earlier studies where salivary CA 125 level showed significant elevation in OSCC than healthy controls [7]. The significant increase in salivary CA 125 may be due to increased shedding of epithelial cells as the normal oral mucosa progresses to a malignant one through premalignant changes. This phenomenon may be a source of false positive result, because as stated earlier, CA 125 is present on the surface of normal cells. This can be explained by the fact that a normal value of serum CA125 is < 30 U/ml, but with normal oral mucosa the salivary CA 125 level may be up to 85 U/ml [7].

The principle mechanisms, by which various molecules enter the body fluids, are the processes of ultrafiltration or transdiffusion. The prognostic utility of serum CA 125 in malignancies other than OSCC suggests that the assessment of CA 125 has a value, only when it is shed in body fluids as a tumor associated antigen [10]. This suggests that perhaps the quantitative assessment of such molecules in serum is more valuable than in saliva.

Previous studies have shown the prognostic usefulness of serum CA 125 in malignancies. However, to the date there are no studies showing serum CA 125 levels in potentially malignant disorders. Oral submucous fibrosis is a potentially malignant disorder with many systemic disturbances. Thus, we selected oral submucous fibrosis among all potentially malignant disorders. However, in current study the difference in serum CA 125 levels between OSF and OSCC was not significant, which suggests that serum CA 125 level shows significant increase when a normal mucosa is subjected to chronic irritation, inflammation or develops epithelial dysplasia. However, whether the same is true when the dysplastic epithelium turns into a malignant one, cannot be concluded. Studies with larger sample size may help to solve this query.

Whether serum CA 125 level elevate as the stage of OSF and OSCC progress is not known and it can be an area for further research. Serum CA 125 level can be a valuable marker for early detection of malignant transformation of OSF. Thus, it can help to select the cases of OSF which show higher propensity for malignant transformation, so that appropriate preventive measures can be instituted in such cases.

Information is not available about serum CA125 in treated cases of OSCC. However, studies suggest that in other epithelial malignancies for e.g. ovarian cancer and breast cancer serum CA 125 level can be a useful marker to monitor the effectiveness of treatment. Similarly, in OSCC patients also, serum CA 125 can be a valuable adjunctive tool for post treatment follow up and to detect recurrences at early stages.

Conclusion

Today the role of CA 125 in oral carcinogenesis seems to be elusive. As per the studies, its level in saliva has been shown to increase in OSCC cases than healthy controls. In our study the value of serum CA 125 level is observed to be increased in OSF compared to healthy controls and in OSCC compared to healthy controls. This suggests that serum level of CA 125 is increased during progression of a normal oral mucosa to potentially malignant disorder or malignancy.

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Table 1 Group 1 (Oral submucous fibrosis)

Sr. No.	Age in years	Gender	Serum CA 125 (U/ml)
1	45	Female	20.7
2	55	Male	12.5
3	50	Male	25
4	50	Male	7.6
5	40	Male	7.4
6	22	Male	9.2
7	57	Male	4.6
8	35	Male	9.5
9	30	Male	12.8
10	28	Male	8.2
11	30	Female	6.5
12	30	Male	24.6
13	30	Female	9.2
14	35	Male	8.5
15	20	Male	6.8
16	42	Male	14.3
17	45	Female	5.9
18	52	Female	11.3
19	45	Male	11.7
20	48	Male	17.6
21	58	Male	7.6
22	38	Female	10.6
23	32	Male	11.4
24	35	Male	9.8
25	40	Male	9.4
26	32	Male	6.7
27	28	Male	8.2
28	33	Male	17.1
29	48	Male	15.7
30	36	Female	14.5

Table 2: Group 2 (Oral squamous cell carcinoma)

Sr. No.	Age in years	Gender	Serum CA 125 (U/ml)
1	40	Male	6.9
2	55	Male	4.6
3	46	Female	252.2
4	48	Male	12.2
5	48	Male	16.4
6	52	Female	8.1
7	46	Female	9.3
8	48	Male	6.3
9	50	Female	6.4
10	56	Male	12.5
11	38	Male	11.4
12	35	Female	12.4
13	46	Male	8.4
14	48	Male	2.6
15	38	Male	11.5
16	52	Male	10
17	47	Male	7.3
18	52	Male	23.4
19	48	Female	14.2
20	56	Male	9.4
21	42	Female	10.4
22	52	Male	23.8
23	34	Female	21.6
24	43	Male	10.4
25	37	Male	9.4
26	55	Male	10.9
27	39	Female	12
28	48	Male	10.5
29	55	Female	11.7
30	42	Female	13.5

Table 3: Group 3 (Healthy controls)

Sr. No.	Age in years	Gender	Serum CA 125 (U/ml)
1	19	Male	8.2
2	21	Male	9.8
3	28	Male	9.4
4	43	Male	6.7
5	19	Male	8.4
6	45	Female	7.5
7	28	Female	6.3
8	50	Male	7.4
9	45	Female	8.3
10	30	Female	7.9
11	53	Male	6.9
12	25	Male	7.6
13	30	Male	6.6
14	55	Female	8.7
15	57	Male	8.7
16	47	Male	9.2
17	49	Male	7.4
18	45	Female	7.8
19	32	Female	6.1
20	37	Female	8.1
21	45	Female	7.6
22	35	Female	6.3
23	37	Female	8.8
24	30	Male	8.1
25	41	Male	5.7
26	32	Female	8.3
27	44	Male	6.7
28	26	Female	6.4
29	45	Male	8.9
30	44	Male	9.3

Table 4: Mean serum CA 125 in study groups

	Group 1 (OSF)	Group 2 (OSCC)	Group 3 (Healthy controls)
Mean serum CA 125	11.50	19.32	7.77
Std. Deviation	5.23	44.25	1.09

Figure Legends

Figure 1: Schematic representation of chemiluminescence immunoassay.

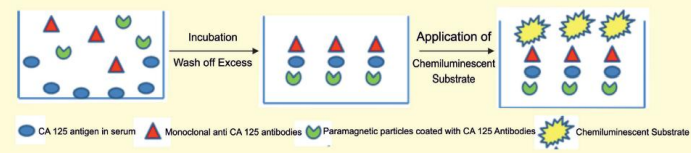

Figure 2: Mean serum CA 125 in group 1, 2 and 3.